be seen exactly what role EMA has in the regulation of E2F-response genes but we suggest that EMA represents the first member of a new class of E2F-like proteins acting to downregulate gene expression in a manner independent of pocket proteins.

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Ribonuclease inhibits Kaposi's sarcoma

Kaposi's sarcoma (KS) is a cancer closely associated with AIDS. Crude, commercial human urinary chorionic gonadotrophin (hCG) preparations have previously been found to inhibit the growth of KS cell lines *in vitro* and in immunodeficient mice[1,2], and to induce tumour regression of KS lesions in AIDS patients[3,4]. Here we report the purification of a ribonuclease (RNase) from a commercial hCG preparation. The pure enzyme killed KS cells *in vitro*, apparently by apoptosis, suggesting that effects of commercial hCG preparations on KS cells and tumours are due, at least in part, to RNase present as a contaminant in these preparations.

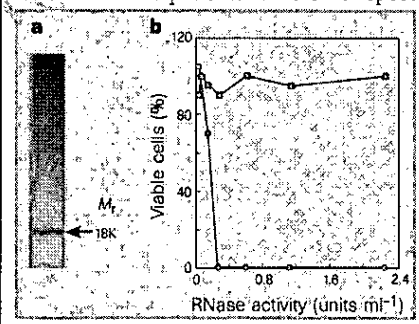

Figure 1 Analysis of RNase from hCG preparations. **a,** Purified RNase (20 ng protein) was subjected to SDS-PAGE on a Phast gel (gradient 10–15%; Pharmacia Biotech) and the gel stained with silver. **b,** Effect of the 18K RNase on viability of KS Y-1 (circles) or HeLa cells (squares). Cells (5×10^4 cells per well, 96-well tray) were incubated with purified RNase (100 µl MDCB medium, Gibco; 16 h; 37 °C) and viability determined with WST-1 reagent (Boehringer-Mannheim).

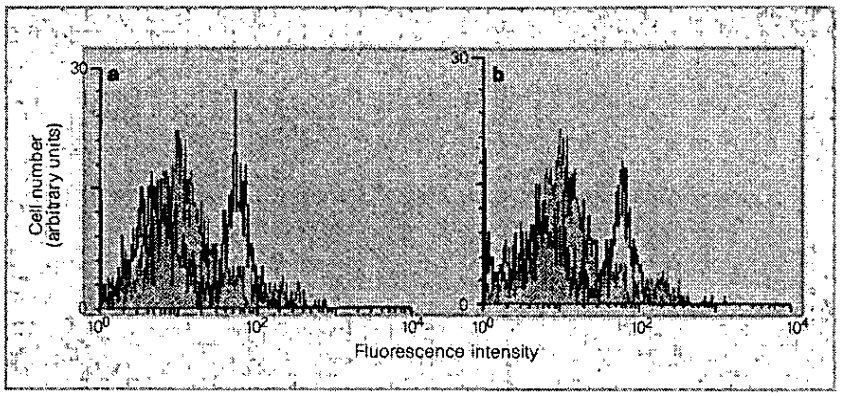

Figure 2 Induction of apoptosis in KS Y-1 cells by the 18K RNase. KS Y-1 cells (5×10^5 cells per well, 24-well tray) were incubated with purified RNase (**a,** 4.46 units RNase activity ml^{-1}; **b,** 1.12 units RNase activity ml^{-1}) in a total volume of 200 µl MDCB medium (16 h, 37 °C) and assayed for apoptosis with the TUNEL reaction (Boehringer-Mannheim). Cells were analysed by FACS; induction of apoptosis (black-enclosed peaks) is shown by an increase in fluorescence intensity attributable to the binding of fluorescent antibody to apoptotic nuclei. Blue peaks are control incubations. Values are percentages of the reading for untreated cells.

We have shown a close association between a human urinary RNase (apparent relative molecular mass (M_r) 18,000) and the β-core fragment of hCG (ref. 5). During the present study we purified the RNase to electrophoretic homogeneity using gel permeation and ribonuclease inhibitor-affinity chromatographies (Fig. 1a; specific activity 892 units per mg of protein (ref. 6)). After electroblot procedures the pure RNase did not crossreact with monoclonal or polyclonal antibodies raised against hCG β-core protein, but it had potent dose-dependent killing activity against a KS Y-1 cell line[1] (Fig. 1b). Furthermore, the RNase induced apoptosis in the KS Y-1 cells in a dose-dependent manner (Fig. 2a). It had no effect on a HeLa cell line (Fig. 1b and data not shown).

Until now, anti-KS activity detected in commercial preparations of chorionic gonadotrophin has been attributed solely to hCG. The present results indicate that an RNase present in these crude preparations makes a major contribution to anti-KS activity. Indeed, the close association of the β-core of hCG with the RNase during purification procedures[5] might explain why partly purified β-core preparations contain particularly potent anti-KS activity. In this regard, recent results obtained by Albini *et al.*[2] must be borne in mind. These authors found that recombinant hCG inhibited growth of KS cells and that the inhibitory effect was blocked by anti-hCG antibodies. It therefore seems likely that at least some of the anti-KS activity detected in commercial hormone preparations is attributable to intact or fragmented hCG. Thus, overall potent anti-KS activity of commercial hCG preparations might result from the combined effects of hCG and RNase activities.

Amino-terminal sequence analysis of the 18K RNase present in human urine indicates that the enzyme belongs to the RNase superfamily that includes onconase[5,7]. Onconase is an amphibian ribonuclease that is cytotoxic to cancer cells *in vitro* and exhibits anti-tumour activity in animal models[8]; it has shown promise in the treatment of human cancers and is currently in phase III clinical trials in patients with pancreatic carcinoma[9,10]. The 18K human RNase might have a similar role in protection against neoplastic disease. It may prove possible to exploit the anti-neoplastic properties of this enzyme as part of a new regime for the treatment of AIDS-related KS and other tumours.

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